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ORIGINAL ARTICLE

Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women

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Abstract
Summary We evaluated the influence of long-term HIV infection and its treatment on distal tibia and radius microstructure. Premenopausal eumenorrheic HIV-positive women displayed trabecular and cortical microstructure alterations, which could contribute to increased bone fragility in those patients.
Introduction Bone fragility is an emerging issue in HIV-infected patients. Dual-energy X-ray absorptiometry (DXA) quantified areal bone mineral density (BMD) predicts fracture risk, but a significant proportion of fracture risk results from microstructural alterations.
Methods We studied the influence of long-term HIV infection on bone microstructure as evaluated by high-resolution peripheral quantitative computed tomography (HR-pQCT) in 22 HIV-positive (+ve) premenopausal eumenorrheic women and 44 age- and body mass index (BMI)-matched HIV-negative (−ve) controls. All subjects completed questionnaires regarding calcium/protein intakes and physical activity, and underwent DXA and HR-pQCT examinations for BMD and peripheral skeleton microstructure, respectively. A risk factor analysis of tibia trabecular density using linear mixed models was conducted.
Results In HIV+ve women on successful antiretroviral therapy (undetectable HIV-RNA, median CD4 cell count, 626), infection duration was 16.5±3.5 (mean ± SD)years; median BMI was 22 (IQR, 21–26)kg/m^2. More HIV+ve women were smokers (82 versus 50 %, p=0.013). Compared to controls, HIV+ve women had lower lumbar spine (spine T-score −0.70 vs −0.03, p=0.014), but similar proximal femur BMD. At distal tibia, HIV+ve women had a 14.1 % lower trabecular density and a 13.2 % reduction in trabecular number compared to HIV−ve women (p=0.013 and 0.029, respectively). HR-pQCT differences in distal radius were significant for cortical density (−3.0 %; p=0.029).
Conclusions Compared with HIV−ve subjects, premenopausal HIV+ve treated women had trabecular and cortical bone alterations. Adjusted analysis revealed that HIV status was the only determinant of between group tibia trabecular density differences. The latter could contribute to increased bone fragility in HIV+ve patients.

Keywords Bone mineral density · High-resolution peripheral quantitative computed tomography · HIV · Osteoporosis · Premenopausal women

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Introduction

Osteoporosis and low bone mineral density (BMD), as well as increased fracture risk, are major complications that are still incompletely understood in individuals with HIV infection treated by antiretroviral therapy [1–4]. Numerous studies have reported increased prevalence of low BMD in HIV-positive (+ve) patients compared to HIV-uninfected persons. The proportion of HIV+ve individuals with osteopenia or osteoporosis in cross-sectional studies ranged from 40 up to 88 % [5]. An important contributor of bone strength is areal BMD (aBMD), expressed in grams per square centimeter and measured at the lumbar or proximal femoral sites using dual-energy X-ray absorptiometry (DXA) [6]. A decrease of one standard deviation (SD) in aBMD doubles the risk of fractures with a continuous risk gradient. Osteopenia and osteoporosis diagnosis are based on an operational definition relying on bone mineral density thresholds [7, 8].

In addition to aBMD, bone microstructure is a key determinant of bone fragility as bone loss is accompanied by a thinning of the spans and by perforations that compromise the overall strength of the supporting tissue [9, 10]. Measurement techniques allow one to analyze the trabeculae in terms of number, thickness, and architectural organization, but are often invasive. High-resolution peripheral quantitative computed tomography (HR-pQCT) is a novel non-invasive tool that allows the analysis of such variables. This instrument is capable of evaluating bone volume, the number, thickness and separation of trabeculae, connectivity, and cortical thickness in vivo, at high resolution (82 μm). Trabeculae in humans are 100–150 μm thick. For the same value of aBMD, subjects with fracture have an altered microstructure, thus emphasizing the importance of this approach to assess bone fragility pathogenesis and fracture risk [11–13]. HR-pQCT has been used in different populations, but not in a particularly fragile population such as individuals living with HIV and treated with antiretroviral agents.

In this study, we aimed to detect differences in areal and volumetric BMD and in cortical and trabecular bone microstructure in a young premenopausal female population with and without HIV infection by HR-pQCT. Our hypothesis was that quantitative bone measurements (DXA) alone do not seem to fully appreciate the increased fracture risk in HIV+ve treated patients. This may have the potential to detect bone disorders at an earlier stage before any mineral mass decrease secondary to sex hormone deprivation of menopause.

Subjects and methods

Study design and population

We conducted a 2:1 case–control study in HIV+ve (n=22) and HIV negative (−ve) age- and body mass index (BMI)-matched premenopausal women (n=44). Cases were HIV+ve Caucasian premenopausal women followed up at the HIV Unit of Geneva University Hospital, Geneva, Switzerland, with a minimum duration of HIV infection of 5 years. Patients had to be on effective antiretroviral therapy (HIV-RNA <40 copies/mL), with or without oral contraception medication. Exclusion criteria were pregnant women, women with amenorrhea, or a history of low energy bone fracture. Control subjects were selected from a cohort of premenopausal women recruited within the Geneva area [14]. Calcium and protein intakes, as well as physical activity, were assessed by frequency questionnaires as previously described [14]. The same questionnaires were used for both cases and controls and administered by the same investigator (a certified dietician). All subjects provided written informed consent before participation. The protocol was approved by the ethics committee of the Geneva University Hospitals.

DXA and HR-pQCT measurements

Bone variables were measured by the same technicians during the whole study period. aBMD (grams per square centimeter) was measured by DXA at the level of the lumbar spine, femoral neck, and total hip by bone densitometers (QDR-4500 and Discovery A; Hologic, Inc, Bedford, MA, USA) with a coefficient of variation (CV) of repeated measurements varying between 1.0 and 1.5 %, as previously reported [15, 16]. The aBMD phantom CV for the DXA case measurement period was 0.34 %.

Volumetric BMD (vBMD) and microstructure were determined at the distal tibia and distal radius by HR-pQCT using an XtremeCT instrument (Scanco Medical, Brüttisellen, Switzerland) as previously described [11, 14, 17]. The following variables were measured: total, cortical, and trabecular volumetric densities expressed in milligram hydroxyapatite (HA) per cubic centimeter; trabecular number, thickness (micrometer), and separation (micrometer); mean cortical thickness (millimeter), cortical area (square millimeter) and porosity (percentage); pores mean and variability size (millimeter); and polar moment of inertia (millimeter to the fourth power). The in vivo short-term reproducibility of HR-pQCT at the distal tibia and distal radius varied from 0.7 to 1.0 % and 0.6 to 1.0 % for bone density, and from 3.0 to 4.9 % and 2.8 to 4.9 % for trabecular microstructure, respectively [14, 17]. The vBMD phantom CV was 0.39 % for the HR-pQCT case measurement period.

HR-pQCT images were filtered (Laplace-Hamming filter; Scanco Medical) and binarized according to the manufacturer's standard instructions. The cortical and trabecular regions were segmented using an automated segmentation method implemented in Image Processing Language (IPL V5.07; Scanco Medical). This method uses two threshold values and a series of morphologic operations (e.g., dilation

and erosion operations) to extract the endosteal and periosteal surfaces of the cortex, which is based on the assumption that the trabecular region is enclosed by a cortical shell. The manufacturer's standard instructions for the use of a Gaussian filter and threshold (filter threshold) were applied also for comparison [18]. Owing to hardware constraints, the Laplace–Hamming filter could not be used on the 19-mm μCT images. Therefore, they were filtered using a Gaussian filter (s¼ 1.2, support ¼2) and binarized using a global threshold of 18.4 % of the maximum value, which is a standard analysis method for μCT images. The cortical and trabecular regions were segmented by semi-automatic, hand-drawn contours around both the endosteal and periosteal surfaces. Cortical porosity was calculated as the number of void voxels in each binary cortex image divided by the total number of voxels in the cortex using IPL. The number of individual pores was counted using component labeling (IPL, Scanco Medical), and the mean pore volume was calculated as the total volume of porosity divided by the pore number [19].

Biochemical determinations

Serum biochemical values were available only for HIV+ve subjects and were determined by the central chemistry laboratory at the Geneva University Hospital (quality assurance # STS 553). HIV-related parameters, such as CD4 cell count, HIV-RNA, and CD4 cell count nadir, were extracted from the Swiss HIV Cohort Study at the closest time point to the measurement visit time (3 months' window). Blood samples were collected between 7 and 10 am after overnight fast for determination of serum levels of creatinine, total alkaline phosphatase (ALP), albumin, calcium, phosphate, bone-specific ALP (bone ALP), 25-hydroxvitamin D, parathyroid hormone (PTH), type 1 collagen amino-terminal telopeptide (P1NP), and CTX (CrossLaps). 25-hydroxyvitamin D3 and PTH were quantified by electrochemiluminescence immunoassays on a Cobas e601 analyzer (Roche, Basel, Switzerland) using Monoclonal Elecsys 25-OH Vitamin D3 (Roche) and Elecsys intact PTH reagents (Roche). Bone ALP was quantified using Acces Ostase immunoenzymatic assay (Beckman Coulter, Fullerton, CA, USA) on a Unicel DxI800 analyzer (Beckman Coulter). The lower limits of detection for these three assays were 10 nmol/L (25-HO-D3), 0.13 pmol/L (PTH), and 0.1 μg/L (bone ALP), respectively.

Statistics

At the time of study conception, we found no published data on distal radius or tibia microstructure (HR-pQCT) for HIV patients. Therefore, the power calculation of this study was computed using literature data on osteopenia in HIV+ve and HIV−ve patients [1, 20, 21]. Cases and controls were matched 1:2 by age (±3 years) and BMI (±0.5 kg/m^2). By including 22 HIV+ve and 44 HIV−ve premenopausal women, we estimated that we would be able to detect a proportion difference of 35 % for patients with osteopenia between the two groups, i.e., 50 % in the HIV+ve group versus 15 % in the HIV−ve group to achieve a power level of 80 % with an alpha threshold of 5 %.

Anthropometric and osteodensitometric variables, demographic and clinical characteristics are given as the median± interquartile range (IQR) by HIV status at baseline (Table 1). Differences between the two groups in bone microstructure variables, lumbar spine, femoral neck and total hip BMD, and Fracture Risk Assessment (FRAX) scores [22, 23] were assessed by univariate conditional logistic regression with an alpha threshold of 5 %. Risk factor analysis (univariate and multivariate) of distal tibia trabecular and distal radius cortical densities in the whole study population (n=66) was first performed using a linear mixed models grouped by pair with an alpha threshold of 5 %. Linear mixed models did not provide additional information as compared to linear regression (likelihood ratio test p values were not statistically significant). HIV status, BMI (kilograms per square meter), age (year), age at first menstrual period (year), prevalent fracture (yes/no), prevalent upper limb fracture (yes/no), tobacco use pack-year unit (PYU) (0–17 PYU versus 18+ PYU), alcohol consumption (<2 units once weekly versus ≥2), logarithm calcium intake (milligrams per day), protein intake (grams per kilogram body weight per day), physical activity (kilocalories per day), and vitamin D supplementation (yes/no) were included in an univariate linear regression model. All variables with p<0.200 in the univariate model were entered in a multivariate linear regression model adjusted for age and BMI to comply with our baseline age- and BMI-matched design, for tobacco use PYU and vitamin D supplementation (yes/no). We assessed the effect of current or past use of tenofovir and boosted protease inhibitor (bPI) on tibia trabecular density and radius cortical density using univariate linear regression. Statistical analysis was performed using STATA software, version 12.0 (StataCorp, College Station, TX, USA).

Results

Comparison of characteristics between HIV+ve women and controls

Twenty-three patients were screened, 22 included in the study (Fig. 1). Baseline characteristics of the 22 cases and 44 controls showed a nonsignificant trend for lower BMI values in HIV+ve women (Table 1). The proportion of active or past smokers was higher in the HIV+ve group compared with the controls (82 versus 50 %,

Table 1 Baseline characteristics

	Premenopausal women		*p*_value
	HIV+ve *N*=22 Median (IQR)	HIV−ve *N*=44 Median (IQR)	
Sociodemographic characteristics			
Age (year)	44.3 (38.9; 46.4)	44.4 (41.3; 46.1)	0.131
BMI (kg/m^2)	21.6 (20.7; 25.9)	22.0 (20.9; 24.8)	0.077
Age at first menstrual period (year)	13.0 (12.0; 14.0)	12.8 (11.7; 14.0)	0.216
Oral contraception [*n* (%)]	22 (100)	42 (96)	0.310
Vitamin D supplementation [*n* (%)]	9 (41)	2 (5)	<0.0001*
Tobacco consumption			
Current or ex-smoker [*n* (%)]	18 (82)	22 (50)	0.013
Pack-years unit	18 (5; 20)	0 (0; 15)	0.146
Alcohol consumption			
Never [*n* (%)]	4 (18)	10 (23)	0.907
1–2 Unit weekly [*n* (%)]	12 (55)	21 (48)	
2 or Unit weekly [*n* (%)]	6 (27)	13 (29)	
Fractures			
Prevalent fracture [*n* (%)]	8 (36)	23 (52)	0.656
Prevalent upper limb fracture [*n* (%)]	2 (9)	9 (20)	0.277
Nutrition and physical activity questionnaire:			
Calcium intake (mg/day)	896.8 (582.9; 1055.0)	792.1 (556.8; 1048.8)	0.830
Protein intake (g/kg BW×day)	0.87 (0.66; 1.03)	0.75 (0.59; 0.87)	0.257
Physical activity (kcal/day)	266.5 (129.7; 358.6)	306.8 (187.0; 418.7)	0.215

IQR interquartile range, *BMI* body mass index, *BW* body weight

*p<0.05

respectively; p=0.013). Calcium intake was similar, whereas protein intake, corrected for body weight and expressed as grams per kilogram body weight×day, was slightly, although not significantly, higher in the HIV+ve group (Table 1). The level of physical activity was 13.1 % lower in the HIV+ve group.

Regarding HIV characteristics, the median duration of infection was 16.5 (IQR,13.5–19.2) years and only included HIV+ve women on successful antiretroviral treatment according to study design definitions [undetectable HIV-RNA; median CD4 cell count, 626 (IQR, 440–831), median BMI, 21.6 (range, 20.7–25.9)kg/m^2]. Current antiretroviral therapy included eight patients (36 %) on boosted protease Inhibitor (bPI) based therapy and ten on non-nucleoside reverse transcriptase inhibitors (NNRTI)-based regimen (45 %); ten patients had a backbone regimen including tenofovir (45 %). 25-Hydroxyvitamin D3 was inferior to target recommendations (>75 nmol/l) in 12 (57 %) subjects (Table 2).

Comparison of bone variable measurements between HIV+ve women and controls

DXA and HR-pQCT values in both groups are presented in Table 3. Figure 2 represents the percent difference (HIV+ve group versus HIV−ve controls) in distal radius and distal

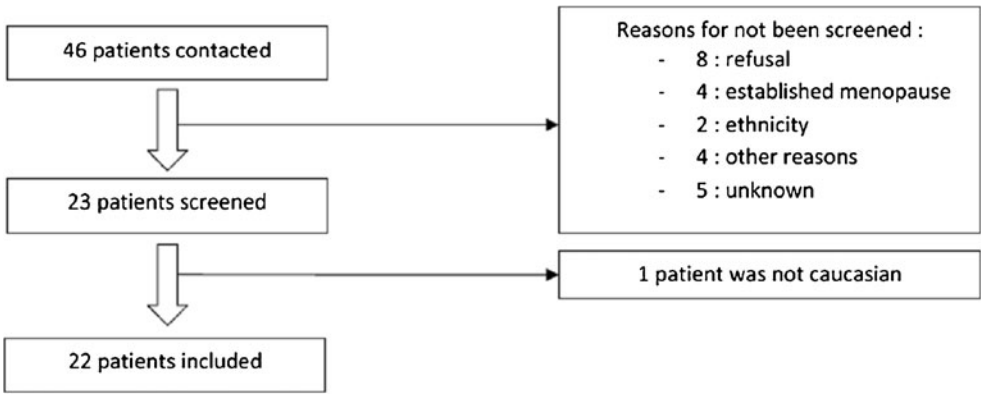

Fig. 1 Study flow chart for the recruitment of HIV+ve women

Table 2 Biochemical parameters and characteristics of HIV +ve subjects

	HIV+ve premenopausal women (N=22) Median (IQR)
Serum parameters	
Adjusted calcium (mmol/l)	
[ref., 2.20–2.52 mmol/l]	2.33 (2.30; 2.36)
Albumin (g/)	
[ref., 35–48 g]	37 (34; 38)
Creatinine (μmol/l)	
[ref., 35–88 μmol/l]	66 (61;71)
25 OH vit. D3 nmol/l <25 [*n* (%)]	2 (10)
25 OH vit. D3 <50 [*n* (%)]	6 (29)
25 OH vit. D3 <75 [*n* (%)]	12 (57)
Bone-specific alkaline phosphatase (μmol/l)	
[ref., 2.9–14.5 μmol/l]	12.3 (8.9; 15.5)
PTH (pmol/l)	
[ref., 1.1–6.8 pmol/l]	4.4 (4.0; 5.9)
P1NP [ref., 15–59 μg/l]	43.6 (35.9; 61.9)
CTX crosslaps [ref., 162–436 ng/l]	396.5 (217.0; 556.0)
HIV characteristics:	
HIV time since diagnosis (year)	16.5 (13.5; 19.2)
HIV C Stade [*n* (%)]	5 (23)
HAART time (year)	11.4 (8.9; 13.5)
HAART [*n* (%)]	22 (100)
Current PI [*n* (%)]	9 (41)
Current boosted PI [*n* (%)]	8 (36)
Current NNRTI [*n* (%)]	10 (45)
Current TDF [*n* (%)]	10 (45)
Others combination [*n* (%)]	3 (14)
CD4 nadir	241 (138; 322)
CD4 cell count	626 (440; 831)
Co- infections status:	
HBV [*n* (%)]	1 (5)
HCV [*n* (%)]	4 (18)

PTH parathyroid hormone, *P1NP* type 1 collagen amino-terminal telopeptide, *HAART* highly active antiretroviral therapy, *PI* protease inhibitor, *bPI* boosted protease inhibitor *NNRTI* non-nucleoside reverse transcriptase inhibitors, *TDF* tenofovir *HBV* hepatitis B, *HBC* hepatitis C

tibia microstructure median pairs. In HIV+ve women, distal tibia trabecular density and number were lower (−14.1 and −13.2 %, respectively) and trabecular separation larger (+15.7 %; p=0.013, 0.029, and 0.037, respectively) compared to controls (Fig. 2). HIV+ve women had also a lower lumbar spine BMD (−6.9 %, p=0.014), but similar femoral neck and total hip BMD. Trabecular density, number, and separation were not different between groups at the radial site. However, a trend towards a lower trabecular density and number in HIV+ve women (p=0.06 for both values) was observed and a −3.0 % lower cortical density (p=0.029) at the radial site (Fig. 2). No differences in cortical porosity were observed between the two groups. The FRAX score (calibrated for Swiss conditions), computed using a BMD-included algorithm, showed a higher probability of hip fracture in HIV+ve women compared to controls.

Effect of specific antiretroviral drug use

Assessment of the relationship between specific antiretroviral drugs and tibia trabecular or radius cortical density did not show any significant effect of current/past use of tenofovir or bPI (data not shown).

Adjusted multivariate analysis

Risk factor analysis of distal tibia trabecular and distal radius cortical densities in the whole study population is presented in Table 4. HIV status was the only risk factor associated with a reduction of tibia trabecular density and of radius cortical density after adjustment for age, BMI, and smoking status. The lower 28.2 mgHA/cm3 tibia trabecular density (p=0.009) in HIV+ve premenopausal women compared to HIV−ve women corresponds to a difference of 41.2 %. The lower

Table 3 Distal tibia and distal radius microstructure (HR-pQCT), lumbar spine and proximal femur BMD (DXA), and FRAX scores in HIV+ve group and in HIV−ve controls

	Premenopausal women		
	HIV+ve, n=22 Median (IQR)	HIV−ve, n=44 Median (IQR)	p value[a]
HR-pQCT tibia:			
Total density (mg HA/cm^3)	282.7 (220.6; 314.1)	298.9 (258.1; 336.4)	0.161
Trabecular density (mg HA/cm^3)	135.0 (112.6; 156.7)	157.1 (144.6; 179.6)	0.013[a]
Trabecular thickness (μm)	71 (63; 77)	70 (65; 82)	0.196
Trabecular number (mm^{-1})	1.58 (1.35; 1.76)	1.82 (1.64; 2.00)	0.029[a]
Trabecular separation (μm)	554 (495; 676)	479 (422; 531)	0.037[a]
Cortical density (mg HA/cm^3)	917.8 (877.0; 931.6)	943.7 (915.5; 965.1)	0.071
Cortical thickness (mm)	1.14 (1.05; 1.30)	1.15 (0.99; 1.26)	0.776
Cortical area (mm^2)	117.6 (103.4; 122.0)	117.8 (102.5; 127.0)	0.764
Cortical porosity (%)	3.43 (2.17; 3.79)	3.54 (2.57; 4.58)	0.631
Pores mean size (μm)	0.18 (0.17; 0.19)	0.18 (0.17; 0.20)	0.359
Pores variability size (μm)	0.08 (0.07; 0.09)	0.08 (0.07; 0.09)	0.482
Polar moment of inertia (1/mm^4)	20625.69 (16662.97; 21291.98)	20014.52 (17435.15; 22574.24)	0.591
HR-pQCT radius:			
Total density (mg HA/cm^3)	319.00 (262.20; 381.90)	343.05 (309.85; 382.05)	0.228
Trabecular density (mg HA/cm^3)	130.10 (108.90; 168.40)	158.05 (138.95; 179.40)	0.062
Trabecular thickness (μm)	63 (55; 69)	66 (61; 71)	0.147
Trabecular number (mm^{-1})	1.76 (1.41; 2.11)	1.94 (1.81; 2.13)	0.068
Trabecular separation (μm)	513 (402; 642)	443 (398; 484)	0.089
Cortical density (mg HA/cm^3)	922.50 (882.90; 948.90)	951.30 (928.00; 977.90)	0.029[a]
Cortical thickness (mm)	0.80 (0.73; 0.98)	0.82 (0.75; 0.94)	0.961
Cortical area (mm^2)	53.40 (49.00; 64.20)	55.55 (52.35; 62.35)	0.717
Cortical porosity (%)	1.13 (0.76; 1.39)	1.00 (0.78; 1.28)	0.123
Pores mean size (μm)	0.15 (0.14; 0.16)	0.15 (0.14; 0.16)	0.916
Pores variability size (μm)	0.06 (0.05; 0.07)	0.06 (0.05; 0.06)	0.861
Polar moment of inertia (1/mm^4)	3853.42 (3473.34; 4464.10)	4034.60 (3650.07; 4956.19)	0.204
DXA scan:			
Lumbar spine BMD (g/cm^2)	1.000 (0.820; 1.077)	1.074 (1.010; 1.170)	0.014[a]
Femoral neck BMD (g/cm^2)	0.776 (0.624; 0.898)	0.813 (0.729;0.879)	0.320
Total hip BMD (g/cm^2)	0.899 (0.761; 1.021)	0.940 (0.834; 0.976)	0.652
T-scores:			
Lumbar spine T-score	−0.70 (−2.33; −0.00)	−0.03 (−0.61; 0.85)	0.014[a]
Total hip T-score	−0.35 (−1.48; 0.65)	−0.02 (−0.88; 0.28)	0.652
Femoral neck T-score	−0.66 (−2.03; 0.44)	−0.33 (−1.09; 0.27)	0.320
Osteopenia			
[T-score ≤1 and T-score ≥2.5] (%)	7 (32)	10 (23)	0.241
Osteoporosis [T_score <−2.5] (%)	1 (5)	0 (0)	
FRAX fracture probability[b]:			
% Major osteoporotic fractures	4.9 (2.6; 5.9)	4.4 (2.8; 5.4)	0.326
% Hip fracture	0.4 (0.0; 2.1)	0.1 (0.1; 0.4)	0.029[a]

IQR interquartile range, *BMD* bone mineral density, *FRAX* refers to the WHO released FRAX tool to assess fracture risk based on a clinical and BMD-based score

[a] p_values were obtained from univariate conditional logistic model

[b] FRAX score 10-year fracture risk probability was computed with femoral neck BMD values

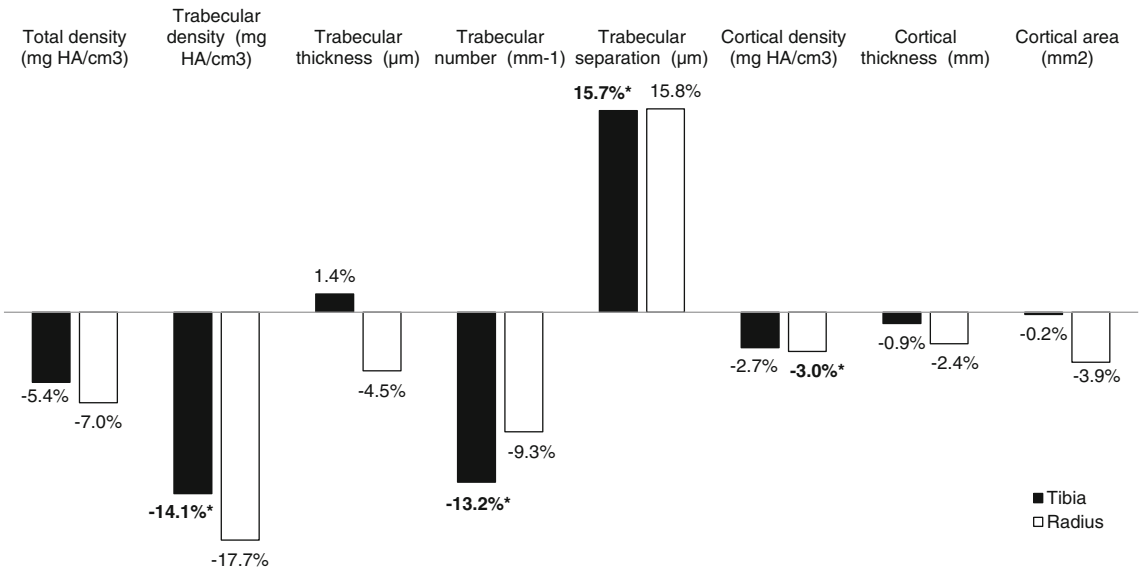

Fig. 2 Percent difference (HIV+ve group versus HIV−ve controls) in distal radius and distal tibia microstructure (HR-pQCT) median pairs in premenopausal women. *p=<0.05 obtained from univariate conditional logistic model which compared HIV+and the control group

31.1 mg HA/cm^3 cortical density (p=0.015) in HIV+ve women corresponds to a difference of 3.5 %.

Discussion

We investigated the influence of long-term, successfully treated HIV infection on bone microstructure as evaluated by HR-pQCT in premenopausal women compared with age- and BMI-matched healthy controls. We observed structural differences between HIV-infected women (with selective structural bone alterations and lower lumbar spine BMD) and HIV−ve premenopausal women.

Bone fragility is frequent in HIV-infected individuals. A meta-analysis of cross-sectional studies conducted between 1996 and 2005 showed that 15 % of HIV-infected individuals had osteoporosis and 52 % had osteopenia. Seropositive individuals were 6.4 times more likely to have low BMD and 3.7 times more likely to have osteoporosis than seronegative controls [1]. Several analyses have identified risk factors predisposing seropositive individuals to low BMD and increased fracture risk. Some of these correspond to those observed in the general population (vitamin D deficiency, low BMI, smoking); vitamin D deficiency is highly prevalent among HIV+ve individuals and has been associated with the use of certain drug classes, namely non-nucleoside reverse transcriptase inhibitors [24]; other factors are related to HIV infection itself, such as HIV-RNA replication and low CD4 cell count. Use of antiretroviral therapy has been implicated and, at least at treatment initiation, HIV-infected patients experience a rapid decrease in BMD [25]. Recent reports have suggested that the overall risk of fracture in treated versus non-treated individuals was reduced [26], but large cohort studies have suggested a negative association of specific antiretroviral drugs, such as tenofovir or boosted lopinavir [4]. To assess fracture risk, DXA instruments are now widely available in many countries, but this technique is not routinely recommended for premenopausal women, regardless of HIV status [27]. However, DXA scans have also limitations, and a significant proportion of fracture risk cannot be explained by BMD alone. Bone structural parameters appear to have an impact on bone strength that is independent of BMD [11–13]. Indeed, differences in bone structural parameters in fractured subjects and controls have been observed for the same level of aBMD in HIV−ve women [11, 28, 29].

In our study, we observed a significant alteration in BMD and bone microstructure among premenopausal HIV+ve women, with a 14.1 % lower tibial trabecular bone density compared to controls. After adjustment for the most common bone health variables, such as BMI, age, smoking status, and vitamin D supplementation (yes/no), this effect on trabecular variables was significant at the tibia site; being HIV positive was the only explanatory variable for the 41.2 % decrease in distal tibia trabecular density in the adjusted analysis. No such difference was found on the distal radius trabecular density. Two factors may explain this finding. First, the technique might be less prone to movement artifacts during tibia image acquisition compared to radius acquisition. Second, tibia is a weight-bearing bone and is more prone to be associated with early structural changes in trabecular bone. However, differences in cortical density were observed at both sites with close to a significant p value at the tibia site and a significant p value at radius sites, but no significant differences in cortical

Table 4 Risk factor analysis of distal tibia trabecular density and distal radius cortical density in both groups (n=66)

	Tibia trabecular density				Radius cortical density			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Coefficient (95 %CI)	p_value	Coefficient (95 %CI)	p_value	Coefficient (95 %CI)	p_value	Coefficient (95 %CI)	p_value
Sociodemographic characteristics								
HIV status : HIV+ve versus HIV−ve	−26.65 (−44.60; 8.69)	0.004*	−28.17 (−49.19; −7.16)	0.009*	−31.51 (−53.04; 9.97)	0.005*	−31.07 (−55.77; −6.36)	0.015*
BMI (kg/m^2)	−0.18 (−3.40; 3.04)	0.911	0.83 (−2.36; 4.01)	0.605	2.35 (−1.42; 6.11)	0.217	−0.06 (−2.99; 2.86)	0.966
Age (year)	1.75 (−0.76; 4.25)	0.169	1.65 (−0.81; 4.10)	0.185	−0.31 (−3.35; 2.74)	0.840	2.96 (−0.79; 6.71)	0.119
Age at first menstrual period (year)	−3.50 (−9.26; 2.26)	0.229			−5.01 (−11.78; 1.76)	0.144		
Risk factors								
Medical history of fracture:								
Yes versus no	14.99 (−2.70; 32.68)	0.095			−12.02 (−33.29; 9.25)	0.263		
Medical history of upper limb fracture:								
Yes versus no	2.23 (−21.99; 26.44)	0.855			−19.92 (−48.10; 8.26)	0.163		
Smoking pack-year:								
18 PYU or more versus 0–17 PYU	2.05 (−17.09; 21.19)	0.832	10.96 (−8.28; 30.19)	0.259	−2.93 (−25.87; 20.01)	0.799	8.61 (−14.11; 31.33)	0.451
Alcohol consumption:								
≥2 units once weekly vs <2	−2.25 (−22.18; 17.67)	0.822	−1.87 (−27.95; 24.21)	0.886	11.27 (−12.16; 34.70)	0.340	−11.90 (−43.23; 19.44)	0.450
Log [calcium intake (mg/j)]	8.63 (−9.11; 26.37)	0.335			0.01 (−0.01; 0.03)	0.287		
Protein intake (g/BW/j)	4.73 (−24.86; 34.31)	0.751			−9.10 (−45.47; 27.27)	0.619		
Physical activity (kcal/day)	0.01 (−0.04; 0.07)	0.639			0.06 (−0.00; 0.13)	0.063		
Vitamin D supplementation: Yes vs no	−16.71 (−40.57; 7.14)	0.166			−28.68 (−57.54; 0.17)	0.051		

CI confidence interval, *BMI* body mass index, *BW* body weight

*p<0.05

porosity were observed. All aspects of bone microstructure were altered in HIV+ve successfully treated premenopausal women. The evaluation of bone microstructure changes using the same technique in hemodialysis patients has shown relative changes to a similar extent in both radius and tibia sites (29 % reduction in total and trabecular density with a trabecular number reduced by 25 %) [30].

Our findings raise certain questions. Would an earlier detection of these differences be useful if targeted to this vulnerable population? Would these noninvasive methods for assessing bone quality have a better discriminative power compared to standard aBMD determination by DXA? Studies in men and women suffering from late-stage chronic kidney disease do not tend to support this hypothesis as BMD by DXA had the same ability to discriminate fracture status as HR-pQCT with no further improvement of fracture discrimination when adding HR-pQCT data to BMD [31]. Although lumbar spine DXA values were lower (−6.9 %) in HIV+ve women in our study, such a small difference would probably not warrant a therapeutic intervention. HR-pQCT alterations are not yet validated to precisely assess fracture risk. Therefore, these two techniques should be considered as complementary.

Our study has several limitations, such as a small sample size, a cross-sectional design, and the lack of biochemical data in controls, especially the absence of vitamin D plasma levels in controls. However, both HIV+ve and control women had similar careful assessment performed by the same investigator, thus adding confidence in the strength of our data. In addition, our data cannot be extrapolated to women with an uncontrolled HIV disease. We believe that prospective data would be welcome to confirm the results of our study. Longer follow-up of these cohorts would be needed to assess the exact fracture risk in women harboring early structural changes, such as HIV+ve women. In the meantime, fracture risk can be decreased by a careful assessment and correction of non-HIV-related factors, such as vitamin D deficiency (57 % of our cohort had values lower than the optimal 75 nmol/L threshold), alcohol and cigarette consumption, or appropriate dietary intakes.

In conclusion, HIV+ve premenopausal women had microstructural bone alterations and lower lumbar spine BMD. These differences could all contribute to a higher bone fragility in HIV+ve patients, and further research is warranted in this population to determine if premenopausal screening might be beneficial to ensure early preventive treatment and maintain quality of life in later years.

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Conflicts of interest None.

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